Responses of North American and New Zealand strains of Holstein-Friesian dairy cattle to homeostatic challenges during early and mid lactation

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Abstract

This study investigated the physiological basis of differences in nutrient partitioning between the North American (NA) and New Zealand (NZ) strains of Holstein Friesian cattle by determining the responses to homeostatic challenges at two stages of lactation. Glucose tolerance tests, epinephrine challenges, and insulin challenges were carried out on consecutive days commencing on day 32 ± 0.48 (mean ± s.e.m) of lactation (T1) and again commencing on day 137 ± 2.44 of lactation (T2). The insulin and non-esterified fatty acid (NEFA) responses to glucose infusion did not differ between the strains. The NZ strain had a greater clearance rate (CR) of glucose (2.04 vs. 1.66 % / min) and tended to have a shorter (34.4 vs. 41.1 min) glucose half-life (t½) at T2 when infused with glucose. The NA cows had a greater glucose response to epinephrine infusion across T1 and T2, and tended to have a greater insulin response to epinephrine infusion. Plasma NEFA concentration declined to similar nadir concentrations for both strains at T1 in response to insulin, though from a higher basal concentration in NA cows, resulting in a greater (-2.29 vs. -1.38) NEFA area under the response curve (AUC) for NA cows. Glucose response to insulin varied with time, tending to be greater for NA at T1, but tending to be lower for NA at T2. The results indicated that NA cows had a greater glycogenolytic response to epinephrine, but both strains had similar lipolytic responses. The results also imply that higher basal circulating NEFA concentrations in the NA strain in early lactation were not due to diminished adipose tissue responsiveness to insulin. There were indications that glucose clearance rate was greater in NZ cows in mid-lactation, and may form the basis of increased body tissue accretion during mid- to late-lactation in this strain.

Keywords: Dairy cows, nutrient partitioning, genetic selection; homeostasis.

Introduction

The onset of lactation in the dairy cow represents a large and abrupt rise in nutrient demand. The increased glucose requirements of the lactating mammary gland require marked adjustments in nutrient partitioning and the metabolism of non-mammary tissues. Hepatic gluconeogenesis is increased to meet mammary demands during early lactation, while glucose utilization by adipose tissue and muscle is reduced. These responses are mediated by reduced circulating insulin concentrations, and through a series of coordinated adaptations – orchestrated by increased circulating growth hormone concentrations – to reduce peripheral tissue sensitivity and responsiveness to insulin (Bell and Bauman, 1997). In addition, adipose tissue responsiveness to lipolytic stimuli is markedly increased in early lactation to facilitate body fat mobilization in support of lactation (Bauman, 2000). Catecholamines are important signals to promote mobilization of fuel stores to meet short-term energy needs. Epinephrine is a neurotransmitter in the sympathetic nervous system with powerful lipolytic action, and induces maximum rates of lipolysis in adipose tissue (Sechen *et al*., 1990).

The administration of bovine somatotropin (bST) to lactating dairy cows alters tissue responsiveness to homeostatic signals to increase nutrient partitioning towards milk production (Etherton and Bauman, 1998). Alterations in responsiveness to homeostatic signals may occur through changes in receptor number, binding kinetics or intracellular expression of the signal (e.g. amplification, enzyme activation; Bauman and Elliot, 1983).

Genetic selection for increased milk yield has been associated with an increased ratio of somatotropin to insulin (Bonczek *et al*., 1988), though relative differences are reduced when high producing cows are placed on a higher plane of nutrition (Hart, 1983). Previous investigations have shown an association between genetic merit and changes in insulin secretion in response to a glucose challenge in juvenile dairy bulls (Mackenzie *et al*., 1988), while Kolver *et al*. (2001) reported a greater lipolytic response to an epinephrine challenge for cows of greater milk yield potential. The greater propensity for body reserve mobilization that accompanies genetic selection for milk production may thus result from changes in the set-points for physiological responses to homeostatic signals.

The Moorepark strain comparison study evaluated the milk production, body condition score (BCS), bodyweight and fertility characteristics of the North American (NA) and New Zealand (NZ) strains of Holstein Friesian (HF) cattle managed under a range of pasture feeding systems (Horan *et al*., 2005). The NA strain had been selected for increased milk yield in a confinement environment, whereas the NZ strain had been selected for milk solids production, feed efficiency and survival in a pasture based system with limited concentrate input. Among the principal findings of the study were greater milk production, lower BCS, and inferior fertility performance for the NA strain across the feeding systems. A notable feature of the Moorepark strain comparison study was the greater milk production response to additional concentrate supplementation for NA cows compared to NZ cows, indicating a greater capacity to partition additional ingested nutrients to milk production for NA cows (Horan *et al*., 2005). It also reported that

increasing concentrate supplementation in pasture-based systems is ineffective as a strategy to reduce the extent of BCS loss for NA cows in early lactation. The current study was carried out to test the hypothesis that differences in nutrient partitioning between the NZ and NA strains are the result of altered tissue responsiveness to homeostatic signals.

Materials and Methods

Animals and experimental design

Two groups of 10 spring-calving, multiparous Holstein-Friesian cows were selected from the NA and NZ groups of the Moorepark strain comparison study. The origins and establishment of the experimental groups from which the cows were selected have been previously described by Horan *et al*. (2005). The NA strain was developed by mating the top 50% of cows in Moorepark (based on pedigree index for milk production) with 5 North American Holstein sires, selected as the highest available in Ireland for pedigree index for milk production. The NZ strain were imported as embryos from New Zealand and implanted into Holstein heifers. These NZ embryos were generated by mating high genetic merit New Zealand HF cows with 5 high genetic merit (based on Breeding Worth, the New Zealand genetic evaluation system) New Zealand HF sires. The experimental animals used in the current study were selected as representative of the NA and NZ treatment groups involved in the Moorepark strain comparison study (Horan *et al*., 2005). The genetic merit for milk production of the experimental groups is outlined in Table 1.

Insert Table 1 Here

Animal management and sampling

Cows were housed in a free-stall barn commencing three weeks prior to expected calving date, and trained to use the Griffith Elder feeding system (Griffith Elder Ltd, Bury St Edmunds, Suffolk, UK). Forage mangers were mounted on electronic load cells and concentrates were dispensed through automatic feeders to facilitate measurement of DMI and calculation of energy balance. Cows had ad libitum access to forage, which was fed once daily and offered to allow for feed refusals of at least 5%. Refusals were removed daily.

Glucose tolerance tests, epinephrine challenges, and insulin challenges were carried out at two time periods for each cow. The first series of challenges were carried out on consecutive days commencing on day 32 ± 0.48 (mean ± s.e.m) of lactation (T1), and the second series of challenges were carried out on consecutive days commencing on day 137 ± 2.44 of lactation (T2). Cows were moved to individual tie-stalls during the period when homeostatic challenges were taking place, and were milked in the stall at 0730 h and 1530 h. Feed was removed at least 1 hour prior to the administration of each challenge. The diet offered in the week preceding and during T1 consisted of ad libitum grass silage (L. perenne spp) plus 8kg/d (as fed) of concentrate. The diet offered in the week preceding and during T2, consisted of ad libitum freshly cut grass (L. Perenne spp.) plus 4kg/d (as fed) of concentrate. The daily concentrate allowance was fed in equal amounts at each milking, and consisted of barley 220g/kg; rapeseed meal 210g/kg; beet pulp 200g/kg; maize gluten 170g/kg; soybean meal 140g/kg; vegetable oil 30g/kg, and mineral

mix 30g/kg. The chemical analyses of the forages and concentrate used are detailed in Table 2.

Insert Table 2 here

Mean daily energy balance (EB) for the week preceding administration of homeostatic challenges was estimated as the difference between energy intake and the sum of energy requirements for maintenance and milk production. The French Net Energy system was used, where one UFL is the NE content of 1 kg of air-dry standard barley for milk production (Jarrige, 1989). Solids corrected milk (SCM) yield was calculated using the equation of Tyrell and Reid (1965). On day 1 (Monday) of each time period, i.e. the day before the first homeostatic challenge, cows were weighed to facilitate calculation of dosage rates, and indwelling jugular catheters were fitted to facilitate collection of blood samples and allow intravenous infusion of glucose, epinephrine and insulin. Body condition score (Lowman et al, 1976) was also recorded for each animal on day 1 of each time period.

Administration of homeostatic challenges

The glucose tolerance test was carried out on day 2 of each time period. Cows were infused with glucose (50% wt/vol dextrose solution; Baxter Healthcare Ltd., Norfolk, England) at a rate of 1.5 g glucose/kg of $BW^{0.75}$ via the jugular catheter at 0900 h and immediately flushed with 10 mL saline. Blood samples were collected at –45, –40, –30, –20, –10, –5, and 0 min relative to the start of infusion, and 2.5, 5, 7.5, 10, 15, 20, 30, 45,

60, 120, 150, and 180 min relative to completion of infusion. Mean infusion times (± s.e.m) for NA cows were 495 s ± 45 s and 400 s ± 17 s at T1 and T2, respectively. Mean infusion times for NZ cows were 401 s ± 18 s and 363 s ± 19 s at T1 and T2, respectively.

The epinephrine challenge was carried out on day 3 of each experimental week. Epinephrine acid tartrate (1 mg/ml solution; Phoenix Pharma Ltd., Gloucester, England) was infused at a rate of 1.4 µg/kg BW via the jugular catheter at 0900 h and immediately flushed with 10 mL of sterile saline. Blood samples were collected at −45, −40, −30, −20, −10, −5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 120, 125, and 130 min relative to epinephrine administration. The epinephrine dose chosen was previously reported to result in a maximum lypolytic response (Sechen *et al*., 1990)

The insulin challenge was carried out on day 4 of each experimental week. Bovine pancreatic insulin (I-5500, lot 064K1582, 28.7 USP units/mg; Sigma, Dublin, Ireland) was dissolved in a sterile solution of 0.01 M HCl to generate a 2 mg/ml solution, and this was diluted with sterile saline to a final concentration of 1 mg/ml. The insulin solution was prepared on the evening prior to the insulin challenges, and stored overnight at 4 ºC. Insulin was infused at a rate of 1.0 µg/kg BW via the jugular catheter at 0900 h, and immediately flushed with 10 mL of sterile saline. The blood sampling schedule for the insulin challenge was identical to that of the epinephrine challenge

All blood samples collected during each challenge were decanted into tubes containing 100 international units of heparin, centrifuged at 2000 × g for 15 mins at 4 °C, and the plasma was harvested and stored at -20 °C until analysis.

Laboratory procedures and sample analysis

The DM, NDF, crude fiber and CP of the forage and concentrate samples were analyzed as described by McNamara et al. (2003). Determination of in-vitro dry matter digestibility (DMD) was carried out by near-infrared spectroscopy using a NIRsystems 6500 spectrophotometer (Perstorp Analytical Incorporated, Silver Springs, Maryland, USA). Silage pH was measured on the juice pressed from the silage using a glass electrode and a pH meter (Radiometer pHM2 standard pH meter-radiometer, Copenhagen). The organic matter digestibility of grass was determined as described by Morgan et al. (1994).

Blood plasma was analysed for glucose and NEFA concentrations by enzymatic colorimetry, using an ABX Mira Autoanalyser (ABX Mira, Cedex 4, France) and appropriate kits (NEFA kit supplied by Wako Chemicals, GmbH, Nissanstraße 2, D-41468 Neuss Germany; glucose kit supplied by ABX Montpellier, Cedex 4, France). Plasma insulin was assayed using a solid-phase fluoroimmunoassay (AutoDELFIA, Perkin Elmer Life and Analytical Sciences, Turku, Finland; Perkin Elmer kit no. 312439, supplied by Unitech BD Ltd, Dublin, Ireland). The inter- and intra-assay coefficients of variation were 8.72% and 5.71% respectively. The minimum detectable concentration of the assay was 1.52 µIU/ mL.

Data handling and statistical analysis

Metabolite and hormone responses to each homeostatic challenge were calculated as area under the response curve (AUC), corrected for differences in baseline value. Area under the curve was calculated using the EXPAND procedure in SAS (SAS Institute, 1991). Baseline values for each analyte were determined by calculating the overall mean concentration of samples collected prior to administration and samples collected from 120 min post challenge. When measuring the AUC response to the hormone/metabolite administered (i.e. glucose AUC in response to the glucose tolerance test, and insulin AUC in response to the insulin challenge), calculations were from the time of administration until the time of return to baseline concentration for the individual animal. In all other instances, the AUC was calculated from 0 min until the average time that maximal response had occurred to minimise the effect of counter-regulatory mechanisms. Maximal responses occurred at 20 mins after administration of epinephrine for both glucose and NEFA. For the insulin and glucose challenges, maximum NEFA responses occurred at 30 mins after infusion.

The glucose response to the insulin challenge was expressed as the fractional rate of glucose clearance (FCR). This was calculated as the slope of the natural logarithm of glucose concentration over the initial declining phase (0 to 20 min) plotted versus time.

The half life (t½) and clearance rate (CR) of glucose and insulin were calculated using the NLIN procedure in SAS (SAS Institute, 1991). Data from the first 60 min following infusion of each challenge were fitted to the following equation:

$f(t) = b * e^{(c * t)}$

where

t = time,

b = parameter for starting concentration,

c = parameter for rate of decay.

Clearance rate is the slope of this exponential function. Therefore, CR and t½ of glucose and insulin were calculated as follows:

$CR, \%/min = 100 * (\ln [t_a] - \ln [t_b]) / (t_b - t_a)$

$t½ = \ln (2) / CR$

where

$[t_a]$ = concentration of metabolite or hormone at time a (t_a)

$[t_b]$ = concentration of metabolite or hormone at time b (t_b)

Data from T1 and T2 were analyzed as repeated measures using the MIXED procedure of SAS (SAS Institute, 1991). Treatment, time and a treatment by time interaction term were included in the models as fixed effects; cow was treated as a random variable nested within treatment, and an autoregressive covariance structure was used. The P-values presented in Tables 4-6 represent the main effects of strain, time, and the interaction between strain and time. Pair-wise comparisons of strain effects within time period and time effects within strain were adjusted using the Tukey-Kramer test; where reported in the text of the results section, these comparisons are described using adjusted P-values.

Results

Milk production, EB, BCS and bodyweight

The milk yield, DMI and EB data of the strains during the periods of administration of homeostatic challenges are presented in Table 3. The NA strain had higher milk yield at T1 ($P = 0.02$) compared to the NZ strain. Solids-corrected milk yield did not differ ($P = 0.37$) between the strains at T1 due to a higher milk fat content for NZ cows ($P = 0.02$). Mean daily EB ($P = 0.92$) was similar for the strains at T1, and differences in DMI were not significant ($P = 0.13$). The NA cows had a greater milk yield ($P = 0.01$) and lower milk fat concentration ($P = 0.04$) compared to the NZ strain at T2. Solids-corrected milk yield ($P = 0.04$) and DMI ($P = 0.03$) were greater for NA compared to NZ cows at T2. However, DMI as a percentage of body weight ($P = 0.91$) and daily EB ($P = 0.16$) did not differ between the strains during this time period. The NA cows were heavier ($P < 0.05$) during both experimental periods. There was no difference in BCS between the strains at T1 ($P = 0.46$) or at T2 ($P = 0.13$). The milk production, postpartum EB, and metabolic profiles of the animals used in the current study have been previously reported (Patton *et al*., 2008).

Insert Table 3 Here

Glucose tolerance test

Intravenous infusion of glucose resulted in an acute increase in plasma insulin concentration, and a reduction in plasma NEFA concentrations (Table 4; Figure 1). The

NA and NZ strains had similar glucose AUC (P = 0.73), insulin AUC (P = 0.32), NEFA AUC (P = 0.85), glucose CR (P = 0.37), and glucose t½ (P = 0.93) at T1.

Insert Table 4 Here

Insert Figure 1 Here

The CR of glucose was greater for NZ compared to NA cows at T2 (P = 0.03), while t½ for glucose tended to be greater for NA cows at that time (P = 0.07). There were no differences between the strains at T2 in insulin AUC (P = 0.89), glucose AUC (P = 0.17) or NEFA AUC (P = 0.63). A significant effect of time was observed, where the insulin response to glucose infusion was greater at T2 versus T1 (P < 0.01).

Epinephrine Challenge

Plasma concentrations of glucose were acutely elevated by intravenous infusion of epinephrine at T1 and T2 (Table 5; Figure 2). The NA strain tended to have a greater glucose response to epinephrine at T1 (P = 0.10) and T2 (P= 0.14), as measured by AUC, resulting in an overall greater response (P = 0.04) for NA cows across the two time periods.

Insert Table 5 Here

Insert Figure 2 Here

Epinephrine infusion also resulted in an acute increase in circulating concentrations of NEFA, which returned to baseline concentrations by 45 min post infusion. The NEFA response to epinephrine, measured as AUC, was similar for both strains at T1 ($P = 0.25$) and T2 ($P = 0.73$). The NA cows tended to have a greater ($P = 0.07$) insulin response to epinephrine infusion across the 2 time periods.

Insulin Challenge

The NA cows had a greater NEFA AUC compared to NZ cows at T1 ($P = 0.02$), whereas the strains had a similar ($P = 0.51$) AUC at T2 (Figure 3; Table 6). The NEFA AUC in response to the insulin challenge was greater in both strains at T1 versus T2 (Time effect, $P < 0.01$). The baseline plasma NEFA concentration, measured as the mean concentration from -45 min to 0 min relative to infusion of insulin, was greater at T1 compared to T2 (0.25 vs 0.11 mmol/L; $P < 0.01$).

Insert Table 6 Here

Insert Figure 3 Here

The FCR of glucose in response to the insulin challenge was similar for both strains at T1 ($P = 0.77$) and T2 ($P = 0.45$) (Table 6, Figure 4). Glucose response to the insulin challenge was also measured as AUC. The NA cows tended to have a greater ($P = 0.11$) glucose response area at T1, whereas NZ cows tended to have a greater ($P = 0.13$) response area at T2 (Table 6). This resulted in a significant strain by time interaction for glucose AUC in response to the insulin challenge ($P = 0.04$).The baseline value for

glucose was greater for NA cows compared to NZ cows at T1 (2.94 vs. 2.58 Mmol/L: P = 0.04), whereas both strains had similar baseline plasma glucose concentrations at T2 (3.38 vs. 3.55 Mmol/L; P = 0.30), resulting in a significant strain by time interaction for baseline glucose concentrations during the insulin challenges (P = 0.04).

Insert Figure 4 here

The half-life of insulin following insulin administration did not differ between strains at T1 (P = 0.52) or at T2 (P = 0.15). The CR of insulin was similar for both strains at both T1 (P = 0.66) and T2 (P = 0.22). The CR of insulin tended to be greater (P = 0.08) in both strains at T1 compared to T2.

Discussion

The NA and NZ strains of Holstein Friesian cattle differ considerably in their nutrient partitioning and BCS profiles when managed in a pasture-based feeding system. Previous reports have indicated that these two strains have a comparable rate of BCS loss during early lactation; however NZ cows maintain a higher BCS throughout lactation and have a greater rate of BCS gain post-nadir (Horan *et al*., 2005; McCarthy *et al*., 2007), indicating that NA cows maintain preferential partitioning of nutrients to milk production for a greater duration *post partum*. In the current study, a series of metabolic challenges were carried out to investigate if strain differences in nutrient partitioning were associated with alterations in tissue responsiveness to homeostatic stimuli. The challenges were carried out during wk 4-5 of lactation, and again during wk 19-20 of lactation, to determine

346 whether the responses of the strains to the challenges varied according to stage of
347 lactation and EB status.
348
349 Calculated EB was more negative for both strains at T1 compared to T2 as expected,
350 though differences in EB between the strains were minor during both time periods. Daily
351 milk yield was greater for NA cows at both time periods, whereas solids-corrected milk
352 (SCM) production was similar for both strains at T1 and greater for the NA strain at T2.
353 The NZ cows had higher milk fat concentration, consistent with previous reports from
354 strain comparison studies (Horan *et al*., 2005; Kolver *et al*, 2002). Differences in BCS
355 profiles between the strains were less pronounced in the current study than previously
356 documented (Harris and Kolver, 2001; Horan *et al*., 2005). In the current study, both
357 strains had comparable BCS loss during early lactation (T1), and had similar BCS in mid
358 lactation (T2); however the NZ cows accumulated significantly more body reserves from
359 T2 to the end of lactation (Patton *et al*., 2008).
360
361 Intravenous infusion of glucose resulted in an acute increase in plasma insulin
362 concentrations. The insulin response was similar for both strains at each time period,
363 though both strains had a greater insulin response at T2 compared to T1. This observation
364 is likely a reflection of superior energy balance and reduced mammary glucose demand at
365 T2 compared to T1. In support of this, pancreatic insulin secretion in response to glucose
366 and propionate infusions is greater in non-lactating cows than lactating cows (Lomax *et*
367 *al*., 1979). Similarly, Sano *et al*. (1993) used a hyperglycemic clamp to demonstrate that
368 the increase in circulating insulin concentrations in response to glucose infusion is

reduced in lactating compared to non-lactating cows. Staufenbiel *et al*. (1992) concluded that flow of metabolites to the mammary gland in early lactation was supported by both reduced pancreatic response to insulinotropic stimuli, and decreased responsiveness of peripheral cells to insulin. In any case, there was no evidence of differences in insulin response to an insulinotropic stimulus between the strains in the present study. Chagas *et al.* (2003) showed that New Zealand Friesian cows had a greater insulin response to a glucose challenge than North American Holstein cows when fed a TMR diet, but the opposite occurred on a pasture-only diet. Strain differences in the insulin response to a glucose challenge appear therefore to be dependent on the basal diet (i.e., environment effect).

It is well documented that peripheral tissue responses to insulin are attenuated during early lactation. These tissue-specific adaptations are collectively described as '*insulin resistance*', and include reduced stimulation of lipogenesis in adipose tissue and whole-body oxidation of glucose (Bauman, 2000). The net effect is to increase the availability of glucose in support of mammary glucose requirements. Insulin resistance occurs whenever normal concentrations of insulin produce a less than normal biologic response. This may be due to a decrease in sensitivity to insulin (i.e. a shift in the dose-response curve to the right), a decrease in maximal response to insulin, or a combination of both (Kahn, 1978). Glucose response to the glucose challenge was measured as area under the response curve (AUC), half-life (t½) and clearance rate (CR). While no strain differences were apparent for measures of glucose response at T1, it was found that glucose had a greater CR and shorter t½ in NZ cows at T2. This indicates greater insulin responsiveness in the NZ cows

compared to NA cows in mid-lactation, as insulin resistance is associated with slower CR, longer t½, and a greater AUC for glucose at similar insulin concentrations (Mertz, 1993). Greater insulin responsiveness in the NZ cows in mid-lactation is consistent with accumulation of more body reserves from mid-lactation to the end of lactation (Patton *et al.*, 2008).

Insulin regulates adipose tissue metabolism by suppressing lipid mobilization and increasing rates of reesterification (Brockman and Laarveld, 1986). Sechen *et al.* (1989) reported that bST-treated cows entered negative EB and had increased basal NEFA concentrations compared to control cows, presumably as part of the coordinated responses necessary to support greater milk production. During glucose and insulin challenges, bST-treated cows had greater decreases in plasma NEFA, indicating that bST-treated cows were more sensitive to the antilipolytic effects of insulin (Sechen *et al.*, 1989). Intravenous infusion of glucose resulted in an acute increase in plasma insulin and a decline in plasma NEFA concentration in the current study, the magnitude of which did not vary between strains or time periods. In contrast to the glucose challenge, the NEFA response to insulin administration varied with both genetic strain and time period. Basal concentrations of NEFA were greater at T1 compared to T2, explaining the greater NEFA AUC responses at T1 compared to T2. The higher basal NEFA concentration at T1 compared to T2 is in agreement with observed differences in EB between the time periods. This is also consistent with the reduced NEFA response to the insulin challenge at T2, where basal NEFA concentrations were lower. Following insulin administration at T1, NEFA was reduced to a similar concentration at 30 min post infusion in both strains,

415 indicating that the higher basal NEFA concentration in the NA strain was not due to
416 diminished adipose tissue responsiveness to the antilipolytic actions of insulin.
417
418 There were significant interactions between strain and time period for glucose AUC in
419 response to the insulin challenge, and also for basal glucose concentration during the
420 insulin challenge. The strain with the greater basal glucose concentration had the greater
421 AUC in response to insulin at both time periods, indicating that the magnitude of the
422 response to insulin was dependent on basal concentration. The fractional clearance rate
423 (FCR) of glucose measures the response of insulin-sensitive tissues. No differences were
424 observed in FCR between the strains at either stage of lactation in this study. Mammary
425 tissue utilizes 60-90% of circulating glucose during lactation, and mammary tissue is
426 insulin-insensitive (Bell and Bauman, 1997). Potential strain differences in glucose
427 utilization by insulin-sensitive tissues only affect a small proportion of the total
428 circulating glucose pool, and consequently are difficult to detect. Differences in FCR may
429 be more apparent in later lactation, when cows are in a more positive EB and have a
430 relatively larger proportion of glucose available for tissue accretion (Sechen *et al*., 1990).
431 In the current study, the mean daily EB of the NA and NZ strains were similar; both were
432 in mild NEB at T1 and both were close to neutral EB at T2.
433
434 Norepinephrine is a neurotransmitter that stimulates lipolysis and NEFA release in
435 adipose tissue of ruminants and other species (Himms-Hagen, 1972), and this effect is
436 simulated by epinephrine administration. The effect of epinephrine treatment on adipose
437 tissue mobilization may be determined from the plasma NEFA response profile.

Epinephrine binds to both the β- and α2- adrenergic receptors on adipocytes. The elevated concentrations of plasma NEFA after an epinephrine challenge represents mobilization of fatty acids, the net effect of β-adrenergic induced lipolysis minus fatty acid reesterification. Sechen *et al* (1989) reported a 2.2-fold increase in NEFA response to epinephrine when lactating Holstein cows were treated with bST, demonstrating that adipose tissue mobilization in response to lipolytic stimuli was enhanced by bST treatment. In the current study, NEFA response (i.e. mobilization) to the epinephrine challenges was not affected by strain or stage of lactation. Similarly, Kolver *et al*. (2001) reported no difference in plasma NEFA response to an epinephrine challenge between North American HF and New Zealand HF cows, either in a TMR or pasture-feeding scenario. Their study also reported a trend towards a strain by diet interaction for glycerol response to epinephrine, with pasture-fed North American cows and TMR-fed NZ cows having a greater response. These groups had lower EB than their strain counterparts on opposite diets, which suggested that the degree of lipolytic response to epinephrine, but not NEFA mobilization, was influenced by differences in energy status. The EB of the NA and NZ strains was similar within experimental period in the present study. The NEFA AUC of the strains did not differ between time periods however, indicating that energy status did not influence the NEFA mobilization response to epinephrine.

Epinephrine stimulated an acute increase in circulating glucose concentrations, presumably reflecting increased hepatic glycogenolysis and reduced glycogenesis in both strains. The glucose response to epinephrine infusion was greater in NA cows compared to NZ cows in the current study. In contrast, Kolver *et al*. (2001) found no difference in

glucose response to epinephrine between the North American HF and New Zealand HF strains; however cows fed a total mixed ration (TMR) had a greater response to epinephrine than pasture-fed cows. Sechen *et al*. (1989) also observed an acute increase in plasma glucose after administration of epinephrine, but no difference in response due to bST treatment. As in the current study, Sechen *et al*. (1989) observed that plasma insulin concentration was acutely elevated in response to epinephrine. This insulin response counter-regulates the effects of epinephrine on plasma glucose concentration; the degree of insulin response is determined by the magnitude of glucose increase.

Conclusions

The NA and NZ strains of Holstein Friesian cows exhibited some different responses to acute metabolic challenges that varied with stage of lactation. The greater glucose response to epinephrine of the NA strain indicates enhanced hepatic glycogenolysis and/or reduced glycogenesis. The NA cows also had a greater reduction in plasma NEFA in response to insulin compared to NZ cows in early lactation, due to a higher basal NEFA concentration for NA cows at that time. The NZ cows had a greater glucose clearance rate and shorter half-life than the NA cows in mid-lactation when infused with glucose, indicating that the NA cows may have a greater degree of insulin resistance at that stage of lactation. It is plausible that this plays a key role in the continued preferential partitioning of nutrients to the mammary gland at the expense of body reserve repletion during mid- to late-lactation in the NA cows, whereas NZ cows accumulate body reserves during this period on grass-based diets.

Acknowledgements

The authors would like to thank Mr. J.P. Murphy, Mr. J. Kenneally and the Moorepark farm staff for management and care of the animals. The technical assistance of Mr. T. Condon, Ms. N. Galvin, and Ms. N Hynes is also appreciated. National Development Plan funding is gratefully acknowledged.

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Table 1 *Genetic merit of the North American and New Zealand Holstein Friesian cows based on predicted differences[1] and standard deviations (SD) for milk production, calving interval and survival*

	Strain[2]	
Trait	NA	NZ
Milk (kg)	+ 210 (117)	+ 1 (157)
Fat (kg)	+ 6.2 (3.5)	+ 6.5 (5.0)
Protein (kg)	+ 7.4 (4.4)	+ 3.7 (4.0)
Fat (g/kg)	+ 0.10 (1.4)	+ 1.13 (0.62)
Protein (g/kg)	+ 0.40 (0.32)	+ 0.75 (0.43)
Calving interval (days)	+ 0.99 (1.98)	- 2.86 (1.53)
Survival (%)	+ 0.04 (0.29)	+ 1.14 (0.48)

[1] All predicted differences obtained from the February 2004 international evaluations of the INTERBULL Animal Centre (Uppsala, Sweden).

[2] NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian

Table 2 *Chemical composition of forages and concentrate*[1]

	Grass Silage	Grass	Concentrate
Dry Matter (DM), g/kg	273 ± 53	172 ± 23	871 ± 32
Crude Protein, g/kg DM	117 ± 10	155 ± 31	186 ± 71
NDF, g/kg DM	589 ± 27	390 ± 23	256 ± 20
ADF, g/kg DM	368 ± 23	-	-
Ash, g/kg DM	58 ± 8	79 ± 8	91 ± 3
Starch (g/kg DM)	-	-	182 ± 15
Dry Matter Digestibility[2], g/kg DM	697 ± 40	-	-
Organic Matter Digestibility, g/kg DM	630 ± 33	813 ± 17	-
pH	4.11 ± 0.36	-	-
Net Energy[3,4,5], UFL[6]/kg DM	0.79[3]	1.02[4]	1.14[5]
Net Energy[7], Mcal/kg DM	1.34	1.73	1.96

[1] Values reported are mean ± standard deviation

[2] Estimated using near infrared spectroscopy

[3] The net energy value of silage was related to its *in-vitro* DMD concentration (O'Mara *et al.,* 1997)

[4] The net energy value of grass was determined according to Jarrige (1989)

[5] The net energy of concentrate was calculated from the net energy values for ingredients (Jarrige 1989)

[6] Unité Fourragère Lait, net energy for lactation equivalent of 1 kg standard air-dry barley (Jarrige, 1989)

[7] Estimated based on 1 UFL = 1.7 Mcal/kg (Vermorel, 1989)

628

629 **Table 3** *Milk production, dry matter intake (DMI), bodyweight and energy balance (EB)*
630 *during periods of homeostatic challenges*

Variable	NA[1]	NZ[1]	S.E.D[2]	P-Value
T1[3]				
Milk yield (kg/d)	35.3	30.5	1.40	0.02
Milk fat (g/kg)	45.4	56.0	2.88	0.02
Milk protein (g/kg)	30.4	29.6	0.60	0.41
Solids corrected milk (kg/d)	35.0	33.4	1.24	0.37
Dry matter intake (kg/d)	17.6	15.8	0.88	0.13
DMI (% body weight)	2.99	2.94	0.23	0.80
Energy balance (UFL[4]/d)	-4.72	-4.61	0.87	0.92
Bodyweight (kg)	596	540	19	<0.01
BCS	2.80	2.92	0.16	0.46
T2[3]				
Milk yield (kg/d)	26.1	22.3	0.93	0.01
Milk fat (g/kg)	41.2	46.0	1.85	0.02
Milk protein (g/kg)	32.4	33.3	0.50	0.24
Solids corrected milk (kg/d)	24.9	22.4	1.14	0.04
Dry matter intake (kg/d)	17.2	15.8	0.64	0.03
DMI (% body weight)	2.93	2.94	0.15	0.91
Energy balance (UFL/d)	0.32	0.38	0.72	0.91
Bodyweight (kg)	593	538	24	0.03
BCS	2.46	2.68	0.14	0.13

631 [1] NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian

632 [2] S.E.D = standard error of difference

633 [3] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

634 [4] Unité Fourragère Lait, net energy for lactation equivalent of 1 kg standard air-dry barley (Jarrige, 1989)

635 **Table 4** *Effect of cow strain on responses to intravenous glucose tolerance tests in early*
636 *and mid-lactation*

	T1[1]		T2[1]			*P -values*		
	NA[2]	NZ[2]	NA	NZ	S.E.D[3]	S[4]	T[4]	S x T
Glucose AUC[5]	254	262	258	227	22.1	0.52	0.27	0.18
Insulin AUC	1617^{A}	2195^{A}	3289^{B}	3368^{B}	412	0.42	<0.01	0.55
NEFA AUC	-4.62	-4.88	-3.17	-2.45	1.52	0.81	0.11	0.67
t ½ glucose[6]	36.9	36.6	41.1	34.4	3.59	0.19	0.71	0.22
CR glucose[7]	1.78	1.93	1.66^{a}	2.04^{b}	0.17	0.02	0.96	0.38

637 [1] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk
638 [2] NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian
639 [3] SED = Standard error of difference
640 [4] S = Strain, T = time period
641 [5] AUC = Area under the response curve. Expressed in units of Mmol*min/L for glucose and NEFA and
642 µIU*min/mL for insulin
643 [6] t ½ = glucose half-life (min)
644 [7] CR = clearance rate (%/min)
645 [ABab] Means having different upper case superscripts differ significantly within strain across time period (P <
646 0.05). Means having different lower case superscripts differ significantly within time period across strain
647 (P < 0.05)

Table 5 *Effect of cow strain on responses to intravenous epinephrine challenges in early and mid-lactation*

	T1[1]		T2[1]			*P-values*		
	NA[2]	NZ[2]	NA	NZ	S.E.D[3]	S[5]	T[5]	S x T
Insulin AUC[4]	585	339	753	463	172	0.07	0.17	0.83
Glucose AUC	43.7^{A}	36.8^{A}	32.2^{B}	26.1^{B}	4.11	0.04	<0.01	0.88
NEFA AUC	6.11	5.03	5.14	5.44	0.93	0.56	0.65	0.27

[1] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

[2] NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian

[3] SED = Standard error of difference

[4] AUC = Area under the response curve. Expressed in units of Mmol*min/L for glucose and NEFA and µIU*min/mL for insulin

[5] S = Strain, T = time period

[ABab] Means having different upper case superscripts differ significantly within strain across time period ($P < 0.05$). Means having different lower case superscripts differ significantly within time period across strain ($P < 0.05$)

Table 6 *Effect of cow strain on responses to intravenous insulin tolerance tests in early and mid-lactation*

	T1[1]		T2[1]			*P-values*		
	NA[2]	NZ[2]	NA	NZ	S.E.D[3]	S[4]	T[4]	S x T
NEFA AUC[5]	-2.29^{Aa}	-1.38^{Ab}	-0.69^{B}	-0.42^{B}	0.40	0.01	<0.01	0.33
t ½ insulin[6]	6.27	5.84	5.99	5.04	0.65	0.20	0.18	0.51
CR insulin[7]	6.53	6.82	6.27	5.50	0.64	0.59	0.08	0.24
FCR glucose[8], min	-0.019	-0.019	-0.017	-0.019	0.002	0.49	0.51	0.73
Glucose AUC	-17.0	-12.7^{A}	-17.7	-21.8^{B}	2.68	0.95	0.02	0.04

[1] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk
[2] NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian
[3] SED= Standard error of difference
[4] S = Strain, T = time period
[5] AUC= Area under the response curve. Expressed in units of Mmol*min/L
[6] t ½ = Insulin half-life (min)
[7] CR = clearance rate (%/min)
[8] FCR = Fractional clearance rate of glucose between 0 and 20 minutes after insulin administration. Values represent the slope of the natural logarithm of glucose concentrations (Mmol/L).
[ABab] Means having different upper case superscripts differ significantly within strain across time period ($P < 0.05$). Means having different lower case superscripts differ significantly within time period across strain ($P < 0.05$).

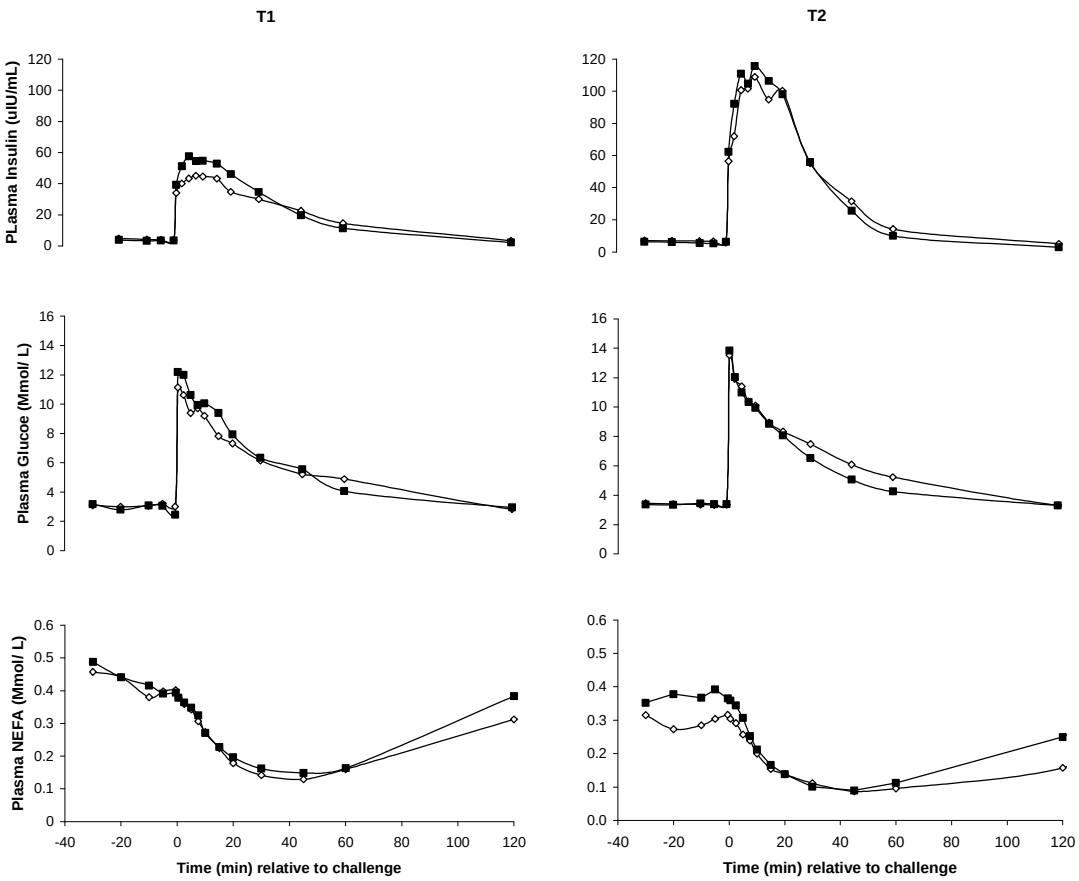

T1
T2
PLasma Insulin (uIU/mL)
Plasma Glucoe (Mmol/ L)
Plasma NEFA (Mmol/ L)
Time (min) relative to challenge
Time (min) relative to challenge

Figure 1

688

689 **Figure 1** Responses of the NA (◊) and NZ (■) strains of Holstein-Friesian cattle to intravenous
690 glucose tolerance tests at 2 stages of lactation (T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2
691 = 137 ± 2.44 days in milk). Cows were infused with 1.5g glucose (50% wt/vol)/kg of $BW^{0.75}$
692 via a jugular catheter. Areas under the response curve and statistical analysis are outlined in
693 Table 4.

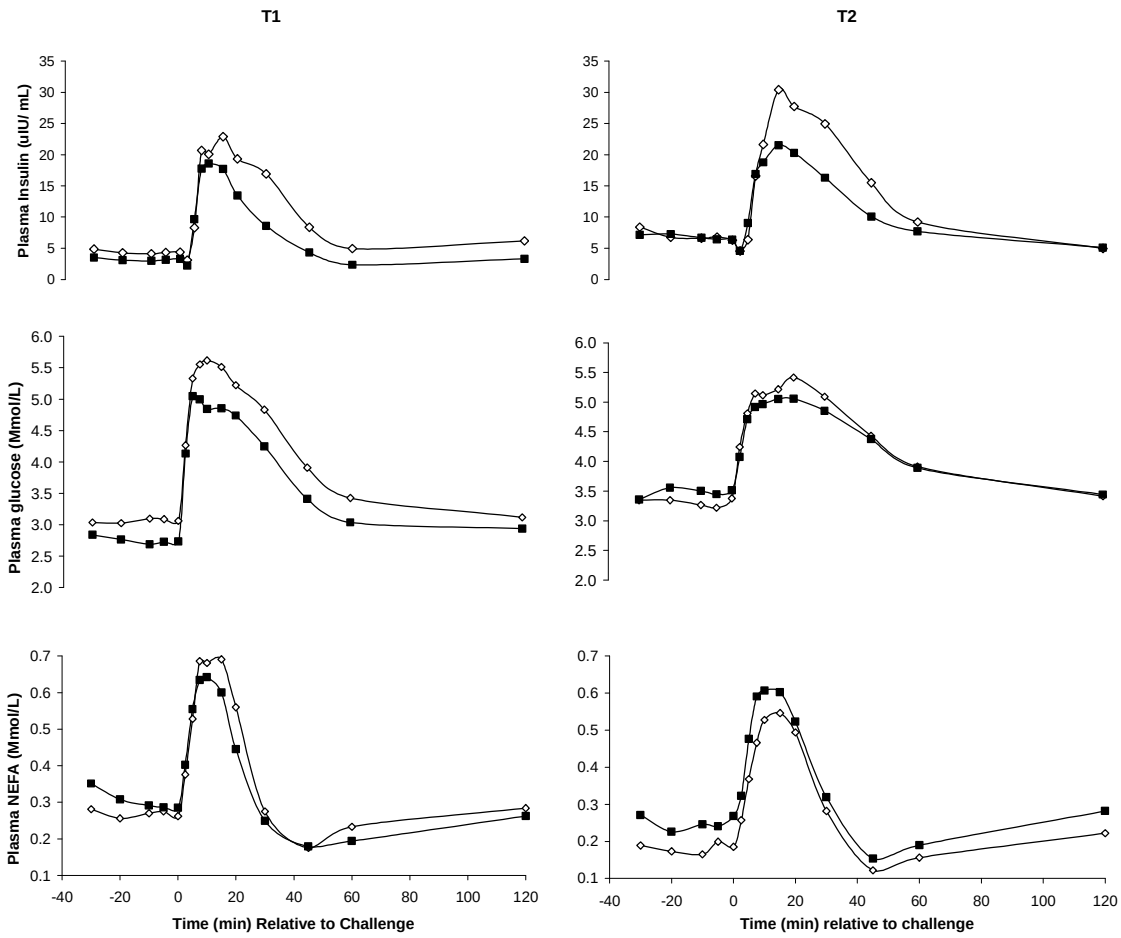

T1
T2
Plasma Insulin (uIU/ mL)
Plasma glucose (Mmol/L)
Plasma NEFA (Mmol/L)
Time (min) Relative to Challenge
Time (min) relative to challenge

Figure 2

Figure 2 Responses of the NA (◊) and NZ (■) strains of Holstein-Friesian cattle to intravenous epinephrine challenges at 2 stages of lactation (T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). Epinephrine acid tartrate (1.4 µg/kg BW) was administered via a jugular catheter. Areas under the response curve and statistical analysis are outlined in Table 5.

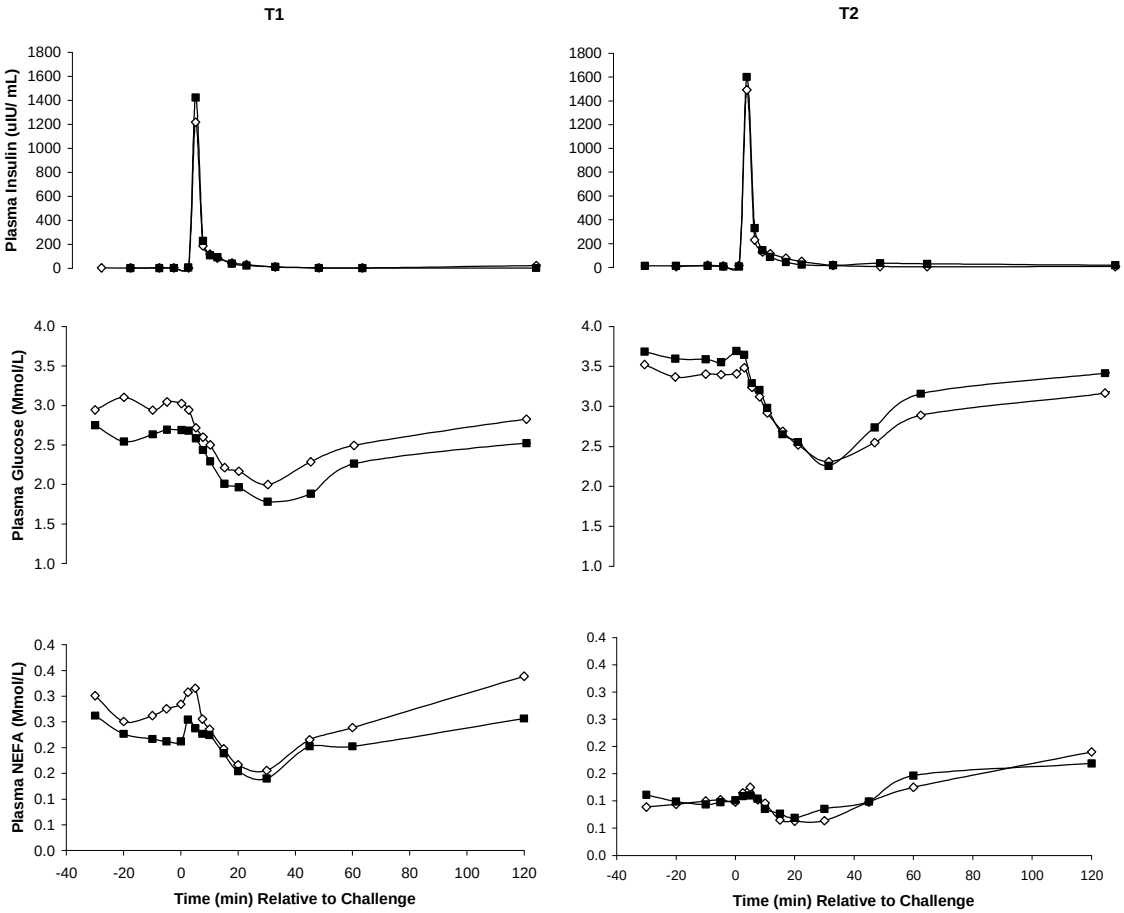
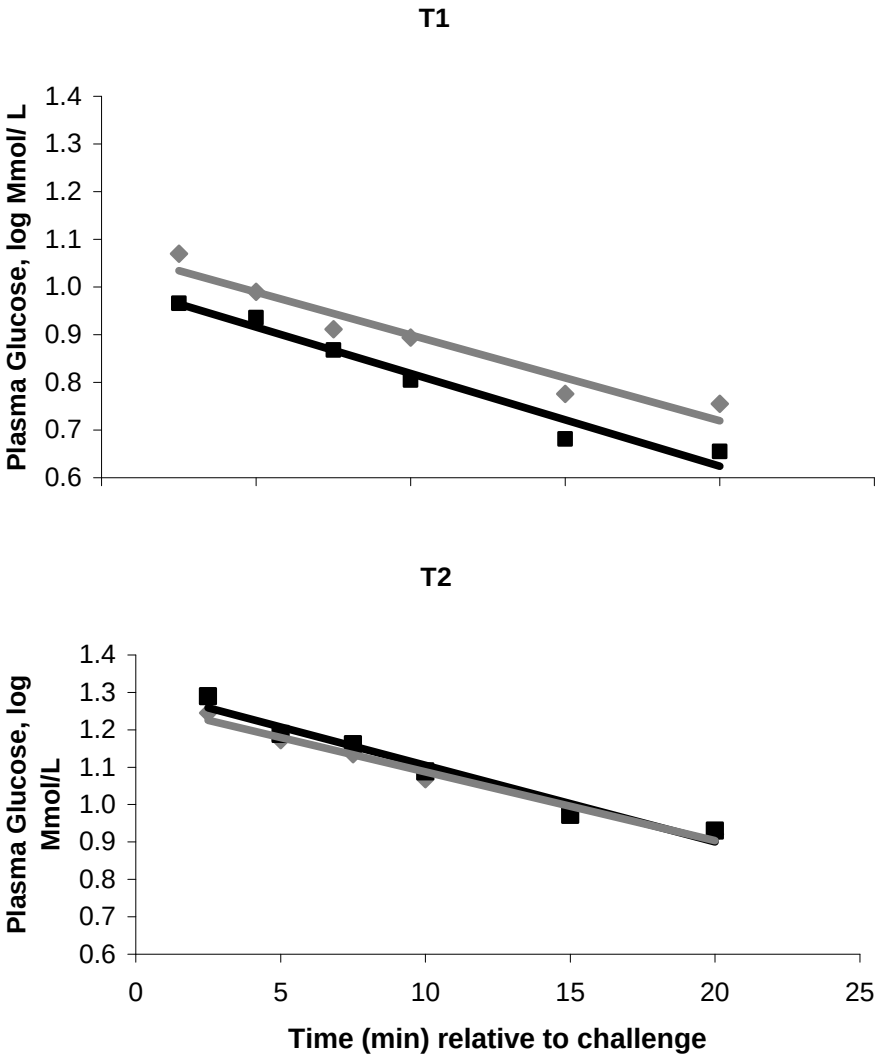

T1
T2
Plasma Insulin (uIU/ mL)
Plasma Glucose (Mmol/L)
Plasma NEFA (Mmol/L)
Time (min) Relative to Challenge
Time (min) Relative to Challenge

Figure 3

728 **Figure 3** Responses of the NA (◊) and NZ (■) strains of Holstein-Friesian cattle to
729 intravenous insulin challenges at 2 stages of lactation (T1 = 32 ± 0.48 (mean ± s.e.m)
730 days in milk; T2 = 137 ± 2.44 days in milk). Cows were infused with 1.0 µg/kg BW of
731 bovine pancreatic insulin, administered via a jugular catheter. Areas under the response
732 curve and statistical analysis are outlined in Table 6.



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743 **Figure 4**

744

745 **Figure 4** Fractional clearance rate (FCR) of glucose in response to intravenous insulin for
746 the NA (♦) and NZ (■) strains of Holstein-Friesian cattle at 2 stages of lactation (T1 = 32
747 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). FCR was calculated
748 as the slope of the natural logarithm of glucose concentration over the initial declining
749 phase (0 to 20 min) plotted versus time. Results are detailed in Table 6. The standard
750 error of mean FCR was 0.002.

751